REMARKS

Status of the Claims

Claims 1-3, 5-8, 10, 12, 14-29, 31-35, and 37-39 are pending in the present application. Claims 4, 9, 11, 13, 30, and 36 were previously canceled. Claims 8 and 14-27 are withdrawn as directed to a non-elected invention. Claims 1, 28, and 29 are amended to specify "and wherein said cytotoxic activity of the expanded cytotoxic lymphyocyte is evaluated as the cytotoxic activity against a target cell labeled with a fluorescent substance by determining fluorescent intensity ascribed to the target cell destroyed by said expanded cytotoxic lymphyocyte." Support for this amendment is found, *e.g.*, on page 34 in the originally filed application. Claims 37-39 are new. New claims 37-39 specify that the fluorescent substance is calcein-AM. Support for this element is found, *e.g.*, in item (3) of Example 1. The claims are amended without prejudice or disclaimer and Applicants reserve the right to claim the canceled subject matter in one or more divisional or continuation applications. No new matter is entered by way of these amendments.

Issues Under 35 USC 103(a)

In the Advisory Action of June 9, 2009, the rejection of claims 1-3, 5, 7, 10, 12, 28, 29, and 31-35 are maintained as allegedly obvious over Davis *et al. J. Immunol.*, 1990,145:785-793, ("Davis") and Cardarelli *et al.*, *Cellular Immunology*, 1991, 135:105-117, (Cardarelli") in view of U.S. Patent No. 5,198,423 to Taguchi *et al.*, ("Taguchi").

The Examiner states that the cited references describe populations of peripheral blood mononuclear cells (PBMCs), *see* Advisory Action. In addition, the Examiner states that Davis teaches a method, which results in an increased number of CD8+ T cells, *see* Advisory Action. According to the Examiner, increasing the number of cytotoxic lymphocytes in a cell population is encompassed by the claim language, which specifies "wherein the expanded cytotoxic lymphocytes maintain cytotoxic activity longer than cytotoxic lymphocytes expanded in the absence of at least one fibronectin fragment", *see* Advisory Action. The Examiner further alleges that since the method of the cited references is the same as that of the instant claims, it would necessarily result in cytotoxic lymphocytes that maintain cytotoxic activity longer then those cultured in the absence of fibronectin.

Independent claim 1, as amended, is directed to a method for expanding cytotoxic lymphocytes which comprises: culturing precursor cells, capable of differentiating to cytotoxic lymphocytes, wherein the precursor cells are selected from the group consisting of peripheral blood mononuclear cells, Natural Killer (NK) cells, umbilical cord blood mononuclear cells, hematopoietic stem cells and blood components containing these cells in the presence of at least one recombinant fibronectin fragment together with interleukin-2, wherein the recombinant fibronectin fragment is a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 1 to 19, wherein said culturing is performed for 2-15 days, wherein the expanded cytotoxic lymphocytes maintain cytotoxic activity longer than cytotoxic lymphocytes expanded in the absence of at least one fibronectin fragment, and wherein said cytotoxic activity of the expanded cytotoxic lymphyocyte is evaluated as the cytotoxic activity against a target cell labeled with a fluorescent substance by determining fluorescent intensity ascribed to the target cell destroyed by said expanded cytotoxic lymphyocyte.

Independent claims 28 and 29, as amended, also specify that the cytotoxic activity of the expanded cytotoxic lymphyocyte is evaluated as the cytotoxic activity against a target cell labeled with a fluorescent substance by determining fluorescent intensity ascribed to the target cell destroyed by said expanded cytotoxic lymphyocyte.

Applicants submit that none of the cited references either alone or in combination teach that the expanded cytotoxic lymphocytes maintain cytotoxic activity longer than cytotoxic lymphocytes expanded in the absence of at least one fibronectin fragment, wherein said cytotoxic activity of the expanded cytotoxic lymphyocyte is evaluated as the cytotoxic activity against a target cell labeled with a fluorescent substance by determining fluorescent intensity ascribed to the target cell destroyed by said expanded cytotoxic lymphyocyte. Instead, the cited references teach that fibronectin increases the *proliferation* of T cells.

Notwithstanding the foregoing, the Examiner indicates in the Advisory Action of June 9, 2009, that increasing the number of cytotoxic lymphocytes is encompassed by maintaining cytotoxic activity longer, as described in the instant claims, since an increase in cell number would also increase the cytotoxic activity. The claims, as amended, clarify that the cytotoxic

activity of the instant claims is related to the potency of the cells rather than to the cell number. That is, the instant claims specify that the cytotoxic activity of the expanded cytotoxic lymphyocyte is evaluated as the cytotoxic activity against a target cell labeled with a fluorescent substance by determining fluorescent intensity ascribed to the target cell destroyed by said expanded cytotoxic lymphyocyte. This element is not taught or suggested in the cited references.

Further, although the Examiner appears to believe that the property of maintaining cytotoxic activity longer than cytotoxic lymphocytes expanded in the absence of at least one fibronectin fragment is inherent in the cited references, obviousness cannot be predicated on what is not known at the time an invention is made, even if the inherency of a certain feature is later established. *In re Rijckaert*, 9 F.2d 1531, 28 USPQ2d 1955 (Fed. Cir. 1993). Accordingly, maintaining cytotoxic activity longer, even if hypothetically inherent, does not render the instant claims obvious over the prior art.

Applicants further submit that the method of Davis or the cells described in Davis are not the same as the claimed method or the precursor PBMCs described in the instant claims. The instant claims specify culturing precursor PBMCs with recombinant fibronectin and IL-2. In contrast, Davis does not teach culturing PBMCs, but instead teaches culturing a cell population having a high-content of T cells. This is evident from Davis, which describes that T cells are isolated, purified and fractionated from prepared PBMCs, *see* Davis, *see* page 786 of Davis, at the section entitled, "Preparation of human peripheral blood T cells", which further states that after the preparation of PBMCs, "T cells were purified...." More specifically, 1) PBMCs are prepared 2) the T cells are isolated, purified, and fractionated from PBMCs, 3) T-cells are prepared, resulting in a cell population having a high-content of T cells, and then the T cells, are incubated with the fibronectin, *i.e.*, the T cells are added to a microtiter well, coated with native fibronectin. In contrast, the present invention concerns the preparation of PBMCs, which are then directly incubated with recombinant fibronectin coated onto a plate. Accordingly, the PBMC cells described in Davis are not encompassed by the precursor PBMCs described in the instant claims. Further, the method of Davis is different from the instantly claimed method.

Based upon the foregoing, the claims are not obvious over the cited references. Applicants respectfully request withdrawal of the rejection.

CONCLUSION

In view of the above amendment and remarks, applicants believe the pending application is in condition for allowance.

Should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact Linda T. Parker Reg. No. 46,046 at the telephone number of the undersigned below, to conduct an interview in an effort to expedite prosecution in connection with the present application.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37.C.F.R. §§1.16 or 1.17; particularly, extension of time fees.

Dated: June 23, 2009

Respectfully submitted,

By_____

Marc S. Weiner
Registration No.: 32,181
BIRCH, STEWART, KOLASCH & BIRCH, LLP
8110 Gatehouse Road
Suite 100 East
P.O. Box 747
Falls Church, Virginia 22040-0747
(703) 205-8000
Attorney for Applicant